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Review

Interaction of Mycobiont: *Piriformospora Indica* with Medicinal plants and plants of Economic importance

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Traditional medicines of plant origin are used by world's large population. Economic development including eradication of poverty in developing countries like, India or other countries of the world required increase in agricultural productivity. Bio fertilizers plays a very important role in modern agriculture, in achieving higher productions in Agriculture at lower input costs using biotechnological innovations at large. An endophytic symbiotic fungus, *Piriformospora indica* isolated from desert soils of Rajasthan, India promotes growth as well as important ingredients of the medicinal as well as economically important plants by forming association with roots of various plants and it has been established as biofertilizer, bioprotector, immunoregulator and agent for biological hardening of tissue culture raised plants. *P. indica* tremendously improves the growth and overall biomass production of a diverse host including legumes, medicinal and economically important plants. Pronounced growth promotional effect was seen with terrestrial orchids. *P. indica* was able to colonize the rhizoids of liverwort and the thalli failed to grow under *in situ* conditions in the absence of this fungus. The fungus also provided protection when inoculated into the tissue culture raised plants by overcoming the 'transient transplant shock' on transfer to the field and renders almost 100% survival on transplant. *P. indica* cell biomass which has potential for promoting growth of many plants (above 145) has been documented so far which include plants such as *Centella asiatica*, *Coriandrum sativum*, *Artimisia annua*, *Spilanthus calva*, *Arabidopsis thalina*, *Cajanus cajan*, *Arachis hypogea*, *Mimosa pudica*, *Cicer arietinum*, *Allium cepa*, *Hordeum vulgare, Zea mays*, *Saccharum officinarum*, *Withania somnifera*, *Solanum lysopersicum,* etc. However, impact of *P. indica* culture filtrate on plant growth promotion has been studied only in few plants. Important medicinal plants and plants of economic importance on which effect of culture filtrate of *P. indica* has been studied include plants such as *Z. mays*, *Bacopa monniera*, *Nicotiana tabaccum*, *Azadiracta indica, Aristolochia elegans, Helianthus annus* and *Solanum melongena*. *P. indica*, a root colonizing fungus which is cultivable axenically, uniquely possesses multifunctional properties such as plant promoter, plant protector, resistance against heavy metals, bio herbicide, immune-modulator, resistance against temperature, salt and stress tolerance as bio fertilizer and tool for basic research. There are prospects that ingredients present in culture filtrate, that are stimulated and produced in response to ingredients of culture filtrate in plants, will be identified in future completely thereby opening more avenues of applications of *P. indica*. Many more properties and functions of *P. indica* cells and culture filtrate are expected to be known in future.

Key words: *Helianthus annus*, *Piriformospora indica*, seed oil content, culture filtrate, aristolochia, plant microbe interaction.

INTRODUCTION

It is felt that one of the major limitations to the development of high quality plant based on medicine is the need for adaptation of both traditional and high-tech agricultural practices to usual species. Yield of medicinal and economic plants need to be defined in biomass, chemical composition and quality. Therefore, new paradigms must be designed that facilitate the investigation of medicinal and economic importance of plant species.

Traditional medicines are used by about 60% of the world's population. These are not only used in developing countries, but also in developed countries as well as where modern medicines are predominantly used. While the traditional medicines are derived from medicinal plants, minerals and organic matter, the herbal drugs are prepared from medicinal plants only. Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine (Li-Sian et al., 2004).

The impact of plant-derived medicine on human history has been remarkable; opium, snakeroot, digitalis, fever bark and chaulmoogra have all left their leaf prints on the human time line (Iwa et al., 1999). There has been a struggle between man and sickness since time immemorial. However, man has acquired methods of treating sickness using his local bicultural environment (Yadav and Patil, 2001). Philosopher Thomas Jefferson wrote "The greatest service which can be rendered by any country is to add useful plant to its culture." Plants have for ever been a catalyst for our healing. In order to halt the trend of increased emerging and resistant infectious diseases, it will require a multi-pronged approach that includes the development of new drugs. Using plants as the inspiration for new drugs provides an infusion of novel compounds or substances for healing disease (Iwa, 1999).

Development of new antimicrobial compounds for resistant organisms is becoming critically important. This situation forced scientists to search for new antimicrobial substances from various sources, such as medicinal plants (Karaman et al., 2003). Plants are valuable source of new natural products. Healthcare systems rely largely on plant material. Much of the world's population depends on traditional medicine to meet daily health requirement, especially within developing countries. Use of plant-based remedies is also widespread in many industrialized countries and numerous pharmaceuticals are based on or derived from plant compounds. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments.

The indigenous inhabitants of various regions throughout the world rely upon plants as a source of medicinal agents to cure many ailments (Robert and Patrick, 1996). Scientists observing these practices have facilitated the discovery of a host of economically important drugs, such as the antihypertensive reserpine, as well as pseudoephedrine, which reduces congestion (Cox and Balick, 1994). Among 250,000 to 750,000 existing plant species, only about 20% have been studied for their medicinal properties. As the resistance towards the present drugs demands to analyze the therapeutic value of more plants, new sciences have been commenced namely pharmacognosy, pharmacology phytochemistry or phytopharmacy.

Indian primitive people used plants for many purposes for instance, medicines for obstetrics and gynecological disorders, antifertility, conception and abortion, to treat leucoderma, leprosy, eye diseases, rheumatism, leucorrhoea, infectious diseases and for skin disease, respectively (Yadav and Patil, 2001). The World Health Organization (WHO, 2000) reported that 80% of the world's population depends upon traditional medicines, herbal remedies and medicinal plants.

Economic development including eradication of poverty in developing country like India or other countries of the world requires increase in agricultural productivity. Increase in production of agricultural products has been experienced in last few decades due to use of high yielding varieties of the seeds and use of chemical fertilizers. However, the increase is insufficient to meet the total needs of ever growing human population. Biofertilizers play very important role in modern agriculture. There are potentials to achieve higher productions in agriculture at lower input costs using biotechnological innovations at large including, biofertilizers. Although chemical fertilizers will continue to serve for increasing crop production in decades to come, the importance of biological sources cannot be ignored (Hardy, 1974). Biofertilizers are important components in modern agriculture, being able to supply different major and minor nutrients through minimal energy consumption as also affecting minimum or zero intervention in the environment and improvement of soil fertility which may have a sustainable positive effect on soil towards productions by crops.

Keeping tremendous potential of biofertilizers, the use of biofertilizers in agriculture is increasing very fast to preserve the quality of soil for ecological reasons and to maintain soil fertility on a sustainable basis. Share of biofertilizers in agriculture is fast increasing. Increase in requirements to produce more food necessitated biofertilizers, their selection, production and application. Gupta (1997) identified two primary reasons behind increasing demand of biofertilizers, which include, increase in crop productivity and necessity to substitute chemical fertilizers with biofertilizers in the interest of soil health and environment.

In India, current annual use of bioferbilizers is around 2200 tons; it is much below the projected requirement (Biswas et al., 1994). However, there is a general consensus that India has great potential to increase the use of biofertilizers. To popularize the use of biofertilizers, concerted efforts right from working out realistic demand estimates, delineation of areas which require inoculation on a priority basis, development of suitable production technology and streamlining distribution channels to co-coordinating training activities are needed. A number of

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Abbreviation: AM fungus, Arbuscular mycorrhiza fungi; **IAA,** Indole-3-acetic acid.

biofertilizers are available in India.

Piriformospora indica Is a wide-host root, colonizing endophytic fungus which allows plants to grow under extreme physical and nutrient condition. It functions as a plant promoter and biofertilizer in nutrient deficient soils, as a bioprotector against biotic and abiotic stresses including root and leaf pathogens and insect invaders, inducing early flowering, enhanced seed production and stimulation of active ingredients in plants. Positive increments are established for many plants of medicinal and economic importance.

Present review is aimed to summarize current state of art of interaction of *P. indica* with plants particularly medicinal plants and plants of economic importance with special reference to the effect of culture filtrate of *P. indica* on economically important plant, *Helianthus annus* and medicinally important plant, *Aristolochia elegans*.

PLANT GROWTH PROMOTING MYCOBIONT: *P. indica*

Despite the numerous important role and ecological function of AM fungus, mass pure inoculum production and axenic cultivation of this group of symbiotic fungi have not grown even till date. These fungi cannot grow like any other fungi apart from their host (obligate photosymbionts). Because of the absence of an authentic pure culture, the commercial production is the greatest bottleneck in use and application of mycorrhizal biotechnology at large. However, an endophytic symbiotic fungus *P. indica,* isolated from desert soils of Rajasthan, India promotes growth as well as important ingredients of the medicinal as well as economically important plants by forming association with roots of various plants and it has been established as biofertilizer, bioprotector, immunoregulator and agent for biological hardening of tissue culture raised plants.

It was Professor Varma and his collaborators, from the School of Life Sciences, Jawaharlal Nehru University, New Delhi, who screened a novel endophytic root colonizing fungus which mimics the capabilities of a typical AM fungus, however, the unique feature is that this fungus is axenically culturable and this is a golden lining of AM fungi for the scientist dealing with the mycorrhizal research. The fungus has been named as *P. indica* based on its characteristic pear shaped chlamydospores, related to the Hymenomycetes of the Basidiomycota (Verma et al., 1998). Interestingly, the *P. indica* has potency to grow axenically on a number of complex and semi-synthetic media (Hill and Käfer, 2001; Pham et al., 2004b; Prasad et al., 2005). The mycelium is mostly flat and submerged into the substratum. The hyphae are highly interwoven often showed anastomosis and are irregularly septated. Hyphae are thin walled and of different diameters ranging from 0.7 to 3.5 µm.

From observations, *P . indica* tremendously improves the growth and overall biomass production of a diverse host including legumes (Varma et al., 1999, 2001), medicinal and economically important plants (Rai et al., 2001; Peškan-Berghöfer et al., 2004; Rai and Varma, 2005; Shahollari et al., 2005). Pronounced growth promotional effect was seen with terrestrial orchids (Blechert et al., 1999; Bhatnagar and Varma, 2006). A study suggested that *P. indica* is able to colonize the rhizoids of liverwort and the thalli failed to grow under *in situ* conditions in the absence of this fungus (Varma et al., 2000). The fungus also provides protection when inoculated into the tissue culture raised plants by overcoming the 'transient transplant shock' on transfer to the field and renders almost 100% survival on transplant (Sahay and Varma, 1999, 2000).

On the basis of anatomical and genomic studies, *P. indica* has been attributed to highly evolved Hymenomycetes (Basidiomycetes). The fungus has great potential in forestry, horticulture, agriculture, viticulture and especially for better establishment of tissue culture raised-plants (Singh et al., 2003a). This fungus promises to serve as the substitute of AM fungi to overcome the long-standing enigma of science. The properties of *P. indica* have been patented (Varma and Franken, 1997) (European patent office, München, Germany, patent number 97121440.8-2104, November 1998). The culture has been deposited at Braunsweich, Germany (DMS number 11827) and 18S rDNA fragment deposited with GenBank, Bethesda, USA (AF 014929).

Moreover in older cultures, hyphae were irregularly inflated, showing a nodose to coralloid shape, mostly coenocytic and septa were laid infrequently containing more than one nucleus. Chlamydospores were formed from thin walled vesicle at the tips of the hyphae and appeared singly or in clusters. They were distinctive due to their pear shaped appearance with 16 to 25 µm in length and 10 to 17 µm in width. The cytoplasm of chlamydospores contained 8 to 25 nuclei. Young spores have thin hyaline walls, but at maturity spores, walls thickened up to 1.5 µm, which appeared two layered: smooth and pale yellow. Neither clamp connections nor sexual structures could be observed. The septal pores consisted of dolipores with continuous parenthosomes. The dolipores were very prominent, with a multilayered cross wall. The parenthosomes were in contact with the ER membranes, which were mostly found near the dolipore (Verma et al., 1998). The thickness of spore and hyphae wall was 0.7 and 3.5 µm, respectively, on an average.

It has been observed that *P. indica* like AM fungi, which functions as a bioregulator, biofertilizer and bioprotector, help plants to overcome the water stress (dehydration), delay wilting of the leaves and prolong life-span of callus tissues. Interestingly, the host spectrum of *P. indica* is very much like AM fungi. The fungus colonizes the roots and improves the health, vigor and survival of a wide range of mono-and dicotyledonous plants. This fungus grows on a large varieties of inorganic, organic and polyphosphates and thus serves as a good model organism to study phosphorus metabolism (Malla et al., 2004). The molecular mass of denatured acid phosphatase (ACPase) of *P. indica* was found to be 66 kDa on SDS PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). This fungus mediates uptake of phosphorus from the substratum and it's translocation to the host by an energy-dependent active process, serves as a strong agent for biological hardening of tissue culture-raised plants, protecting them from "transplantation shock", rendering almost hundred percent survival rate on the hosts tested. This fungus is also a potential "bio-control agent" against potent root pathogens. Thus, it displays immense potential to be utilized as biological tool for plant promotion, protection from pests, and for relieving stress conditions such as those caused due to acidity, desiccation and heavy metal toxicity. Hence it may be concluded, that this novel fungus has immense potential for biotechnological applications at large in future.

Also *P. indica* mediates uptake of phosphorus from the medium and translocates to the host in an energy-dependent process. *P. indica* produces significant amounts of acid phosphatases for the mobilization of broad range of insoluble forms of phosphate, enabling the host plant the accessibility of adequate phosphorus from immobilized reserves in the soil (Varma et al., 2000). An active involvement of the phosphatases in the phosphate metabolism of *P. indica* has been observed. The fungus showed prominent acid phosphatase activity in both intra- and extracellular fractions. This was a direct evidence for the involvement of this enzyme in the phosphate metabolism (Sharma, 2000). In recent observation in *Asphodelus fistulosus*, a common weed in Southern Austria, colonisation was moderately sensitive to P supply (reduced from 60% to just over 30% over the range of P used) (Cavagnaro et al., 2003).

Interestingly, *P. indica* colonization highly promotes the plants growth. The tissue culture raised plantlets, when transferred to the natural environmental conditions with *P. indica*, showed a higher survival rate than the uninoculated control plants. The culture filtrate containing the fungal exudate was able to show the same stimulatory effect on the plants (Verma et al., 1998). The exact chemical nature of the stimulatory compound is still not known. Autoradiographic studies with P32 showed that the fungus is involved in transporting the external phosphate to the colonized plant roots (Singh et al., 2002b).

HPTLC analysis of culture filtrate of *P. indica* emphasizes importance of individual component of culture filtrate involved in stimulation of growth and essential ingredients of plants. Many more ingredients present in culture filtrate that are stimulated and produced in response to ingredients of culture filtrate of *P. indica* in plants may be identified and many more properties and functions of *P. indica* cells and culture filtrate will be known in future (Bagde et al., 2010a).

DIVERSE FUNCTIONS AND APPLICATIONS OF *P. indica*

P. indica fungus associates with the roots of various plant species in a manner similar to mycorrhiza and promotes their growth (Varma et al., 1999, 2001; Singh et al., 2002, 2003a; Pešken-Berghöfer et al., 2004; Pham et al., 2004a; Oelmüller et al., 2004, 2005; Shahollari et al., 2005; Deshmukh et al., 2006). The fungus posseses unique properties to act as biofertilizer, bioprotector and immunoregulator. It also plays a key role in protecting roots from insects by increasing the tolerance of the host roots (Varma et al., 1999; Waller et al., 2005). It also promotes antifungal potential of medicinal plant, *Spilanthus calva* due to increase in spilanthol content after interaction (Rai et al., 2004).

It is observed that among the compounds released in root exudates infected with *P. indica,* flavonoids are found to be present. Flavonoids have been suggested to be involved in stimulation of pre-contact hyphal growth and branching (Gianinazzi-Pearson et al., 1989; Siqueira et al., 1991), which is consistent with their role as signaling molecules in other plant-microbe interactions (Giovannetti and Sbrana, 1998). Cell wall degrading enzymes like cellulase, polygalactouronase and xylanase were found in significant quantities both in the culture filtrate and in the roots exudates colonized by *P. indica* fungus.

Also *P. indica* showed profound effect on disease control when challenged with a virulent root and seed pathogen of *Gaeumannomyces graminis* by completely inhibiting the growth of this pathogen. It indicates *that P. indica* acted as a potential agent for biological control of root diseases, however, chemical nature of the inhibitory factor is still unknown (Varma et al., 2001).

Interaction of *P. indica* with broad spectrum of plants

It has been observed that the host spectrum of *P. indica* and AM fungi are very much alike where it has been calculated that AM fungi interacts with almost 90% of the terrestrial plants (Bagyaraj and Varma, 1995; Giovannetti and Sbrana, 1998; Smith and Read, 1997; Varma et al., 1999), however, only limited members of plant community have failed to interact and they belong to the family of Amaranthaceae, Chenopodiaceae, Cyperaceae, Junaceae, Proteaceae or with lupines and Cruciferae, etc. (Denison et al., 2003). A careful perusal of the literature indicates that this statement may not be true (Leake, 1994; Tester et al., 1987). Denison et al. (2003) have emphasized that model systems are also important as a new research tool to understand the co-operation between microbes and the plants. Cruciferae includes the model plant, *Arabidopsis thaliana* that lacks symbiotic interactions such as mycorrhizae and rhizobia, however, most species of plants are normally infected by mycorrhizae, but some plant taxa do not usually form recognizable mycorrhization.

Exceptions are however, those belonging to members of Cruciferae, and some members of Chenopodiaceae and Amaranthaceae (Read, 1999; Varma, 1999; Varma et al., 2001). Literature reports that the members of these groups normally do not accept AM fungi. *In vitro studies*, on *P. indica* and *S. vermifera* sensu recorded that these two symbiotic fungi profusely interacted with the root system of the crucifer plants such as mustard (*Brassica junacea*), spinach (*Spinaceae oleracea*), cabbage (*Brassica oleracea* var capitata) (Kumari et al., 2003) and *A. thaliana* (Pham et al., 2004a,b; Peškan-Berghöfer et al., 2004; Shahollari et al., 2005) (Table 1). It would be useful to assess the non-hosts of AM fungi with respect to their interaction with *P. indica* for its further functional characterization. In order to enlighten the molecular events that promote the root growth, the difference in protein expression was analysed and modification arises due to the interaction with the fungus. Membrane-associated proteins from roots were separated by two-dimensional gel-electrophoresis (2D-PAGE) and identified by electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MS-MS). *P. indica* consists of secondary metabolites like hydroxamic acids (DIBOA, DIMBOA) which acts as natural pesticides (Varma et al., 2001).

However, the mechanism determining the non-host nature of plant species and preventing the establishment of a functional symbiosis, are not known at generic level till date as similar to AM fungi (Giovannetti and Sbrana, 1998). Nevertheless, present knowledge of the sequential fungal development leading to establishment of functional AM symbiosis suggests that the non-host nature of plants lies in their inability to trigger expression of fungal genes involved in hyphal commitment to the symbiotic status.

MEDICINALLY AND ECONOMICALLY IMPORTANT PLANTS

Many reports indicate the effectiveness of traditional herbs against micro organisms as a result; plants are one of the bedrocks for modern medicine to attain new principles (Evans et al., 2002; Chauhan et al., 2010; Sharma et al., 2010). Medicinal plants have always had an important place in the therapeutic armoury of mankind. Up to 80% of population in developing countries is totally dependent on plants for their primary health care and despite the remarkable progress synthetic organic chemistry of the twentieth century; over 25% of the prescribed medicines in industrialized countries derive directly or indirectly from plants (Newman et al., 2000).

The agro-ecosystem is actually an intervened ecological system where crop plants also do not perform satisfactorily. Crops often succumb to the poor nutrient supply conditions despite increasing fertilizer usage. The crops under pressure for high yield these days find difficulty in meeting their total needs. Absolute dependence of crop reduction system in the country on chemical fertilizers has led to plateau development or even decline in productivity for such crops. Reinforcing the nutrient supply system with organic nutrient supply system, particularly through use of biofertilizers offers a way out. Sixteen chemical elements are known to be essential for plants. Except C, H, or O, the thirteen other elements are taken up by plants from the soil. With increase in production, nutrient removal by crops from the soil has increased, over four times putting fourfold pressure on the soil. Although annual chemical fertilizer consumption has also increased, it was much below the annual nutrient removal by the crops resulting in a yearly negative balance between nutrient removal and supply.

To guarantee enough food for all and or food security, either the population growth has to be controlled or fertilizer has to be produced to meet the ever increasing demand for plant protein. Present biofertilizer production is far below the projected demand. Applied work related to biofertilizers has been done in India and abroad on a large scale with legume as well as non legumes. While the biofertilizer technology appears to be relevant to poor nations, sophisticated basic research involving enterprise or money which can improve the ability of crops or microorganisms to get the most out of microbiological processes is being increasingly carried out in India and abroad.

It may be possible to integrate the use of more than one biofertilizer or screen for new and novel biofertilizer agents for increasing crop yields. Integrated use of biofertilizers, organic manures and chemical fertilizers would not only give higher yields but also help to maintain soil health by narrowing down the gap between nutrient removal and supply.

Crop production is gaining importance day by day. Having the area of production more or less fixed, increase in productivity needs a sustained nutrient supply also. Development of a steady supply system in the rhizosphere of the crop plants is an urgent necessity of the day. The deterioration effect of chemical fertilizers on the environment is a concern for some time. Maintenance of environmental health and sustenance of food production by stabilizing natural balance have been accepted as the only enduring method. Thus, the plant nutrition management, use of potential organisms to supplement partially chemical fertilizers in agricultural practices has been recognized to have significant potential for a great many years to come. Despite the potential of microbiological nutrient supply, only a few successful examples are known.

India is traditionally the biggest buyer of fertilizers importing some 200,000 tons of urea and ammonia each annually. Hence keeping in view of the national needs

Table 1. Host of plants interacting with *P. indica.*

Aneura pinguis L. Dumort. (liverwort)	*Cicer arietinum* (gram)
Adhatoda vasica L. syn. (malabar nut)	*Aristolochia elegans*
Daucus carota L. Queen Anne's-lace (carrot)	*Arachis hypogaea* (groundnut or peanut)
Petroselinum crispum L. (curly parsley)	*Medicago sativa* (alfalfa)
Centella asiatica (brahmi booti)	*Glycyrrhiza glabra* (mulbatti or liquorice)
Cuminum cyminum (zeera or cumin)	*Abrus precatorius* L. (ratti)
Foeniculum vulgare (Saunf or fennel)	Mimosa pudica (sensitive plant)
Carum capticum (ajwain)	*Vigna unguiculata* (rajmah or cowpea)
Coriandrum sativum (dhania or coriander)	*Glycyrrhiza glabra*
Artemisia annua L. (chinese wormwood)	*Acacia catechu* (L.f.) willd (black catechu)
Spilanthes calva DC (clove)	*Nilotica* (L.) willd (gum)
Stevia rebaudiana (sweet leaf of paraguay)	*Prosopis chilensis Stuntz sys*. (chilean mesquite)
Calendula officinalis (asteraceae or aster family)	*P. juliflora* (Swartz) DC. (honey mesquite)
Arnica spp. (in asteraceae oraster family)	*Abrus precatorius* L. rosary pea (precatory bean)
Ave*na sativa* (oat)	*Cicer arietinum* L. (chick pea)
Arabidopsis thaliana L. Heynh. (mouse ear cress)	*Delbergia sisso Roxburg* (rosewood)
Cassia angustifolia Senna Patti (gallow grass hemp)	*Curcuma longa* (turmeric)
Cajanus cajan (pigeon pea)	*Lathyrus odoratus* Linn. (sweet pea)
Terminalia arjuna L. (arjun tree/stembark)	*Pisum sativum* L. (aspen)
Tephrosia purpurea L. Pers. (sarphunkha/purpurea)	*Fragaria vesca* (strawberry)
Salvia officinalis (salvia)	*Glycine max* L. Merr. (soybean)
Mentha piperita (peppermint)	*Quercus robur* L. (clone DF 159) (oak)
Allium cepa	*Cymbopogon martinii* staph Van motia (palmarosa)
Allium sativum	*Colchicum luteum*
Chlorophytum borivillianum Baker (musli)	*Aloe vera syn.*
C. tuberosum Baker (mexican orange)	*Zea mays* L. white (maize)
Azadirachta indica A. Juss (neem)	*Coffea arabica* L. (english coffee)
Helianthus annus	*Elettaria cardamomum* (cardamom)
Dactylorhiza fuchsi Druce (soo') (spotted orchid	*Asparagus racemosus*
D. purpurella (Steph's) soo' (lady orchid)	*Populus tremula* L. (aspen)
D. majalis Rchb. f. (broad leaved marsh orchid)	*P. tremuloides* Michx. (clone Esch5) (quaking)
D. maculata L. Verm. (northern marsh orchid)	*Bacopa monniera* L. Wett. (brahmi)
D. incarnata L. Soo' (early marsh orchid)	*Oryza sativa* L. (rice)
Setaria italica L. (thumb millet)	*Secale cereale* (rye)
Sorghum vulgare Burm. fil. (jujube)	*Saccharum officinarum* (Sugar cane)
Hordeum vulgare L. (barley)	*Panicum miliaceum* (common millet)
Secale cereale	*Zingiber officinalis* (ginger)
Sorghum vulgare (jowar)	*Coleus forskohlii*
Triticum aestivum	*Hyoscyamus niger* (henbane)
Zizyphus nummularia Burm. fil. (jujube)	*Datura stramonium* (datura)
Atropa Belladona (bellodana or deadly nightshade)	*Withania somnifera* L. Dunal (winter cherry)
Solanum melongena L. (egg plant)	*Papaver somniferum*
Picrorhiza kurroa (kutki)	*Ruta graveolens* (rue)
Tectona grandis Linn. f. (teak)	*Mentha piperita* L. (peppermint)
Physalis peruviana (raspberry or cape gooseberry)	*Arabidopsis thaliana*
Petunia hybrida (pink flower)	*Beta vulgaris* Linn. (beetroot)
Nicotiana tobaccum L. (tobacco)	*Brassica napus* L. (canadian turnip)
N. attenuata L. (mountain tobacco)	*Aristolochia elegans*
Solanum lycopersicum (tamatar)	*Tecoma radicans*
Tulsi	*Vinca rosea*
Brassica nigra L (mustard)	*Hemidesmus indicus*
Brassica oleracea var. botrytis L. (alif) (broccoli)	*Brassica oleraceae* L. var capitata (cabbage)

Table 1. Cont...

Dianthus caryophyllus L. (carnation)	*Spilanthes calva*
Saraca indica	*Tagetes indica*
Helianthus annus	*Withania somnifera* L. Dunal (winter cherry)
Tectona grandis Linn. f. (teak)	*Solanum melongena* L. (egg plant)
Genetically modified populus	*Linum sp.*
Chlorophytum borivillianum Baker (musli)	*Bacopa monniera* L. Wett. (brahmi)
Tridex procumbens L.	*Solanum nigrum* L.

and international position, it would be most economical to exploit biofertilizers as an alternative. Besides, concentrating on nitrogen and phosphorus nutrients and possibilities of improving growth as well as yield of the crops by other means could be investigated that may include production of vitamins, auxin- like substances, ascorbic acid, Indole acetic acid, indole butyric acid and antibiotics acting against phytopathogens. Some filamentous organisms like the Arbuscular Mycorrhizae (AM) which form symbiotic association with plant roots have the ability to mobilize phosphorus from soil and thereby helping in absorption of P by plant roots.

Economically important plant, *Helianthus annus*

Asteraceae constitutes one of the largest vascular plants families with about 30,000 species and over 1100 genera. Usually, the members of the family exhibit antimicrobial activity (Rai et al., 1999). Sunflower is cultivated in many places throughout the globe for its high oil content commercially. Commercial varieties contain 39 to 49% oil. Sunflower seeds were the third largest source of vegetable oil world wide following soybean and palm. Sunflower oil is used in certain paints, varnishes and plastics due to its semidrying properties without colour modification associated with oil high in linolenic acid. Also oil is used in manufacture of soaps, and detergents. It is also used as a pesticide carrier, and in the production of agro-chemicals, surfactants, adhesives, plastics, fabric softeners, lubricants and coatings.

Medicinally important plant, *Aristolochia elegans*

Aristolochia species are found all over the world. *A. elegans* is a native to South America, Brazil being its home territory. Among its features are: Beautiful foliage, unusual flowers, freedom from pests and ease of growth make this one favourite vines. The flowers make a great conversation piece looking like something out of a Star Trek episode.

Aristolochia, a neat little vine is a tender evergreen vine with very unusual flowers and beautiful bright green heart shaped leaves. These are about 7.6 cm long by 5 cm wide and grow closely together to create a dense mass of foliage. Slender woody stems twine gracefully in tight coils around fence wire and other supports lifting itself to heights of 3 to 4.6 m. Species of *Aristolochia* are generally called pipe vines or Dutchman' pipes for this reason.

Members of the genus *Aristolochia* are also called birthworts and are occasionally encountered in herbal preparations as a remedy for various ailments as well as to ease the pain of childbirth. They were sometimes used to treat malaria and other diseases. Many *Aristolochia* species contain the alkaloid aristolochic acid and other components. All of these plants are highly toxic, especially to kidneys. Incorrect doses can cause vomiting, pain and even death. Species of *Aristolochia* have been cultivated for use in folk medicine (Kimura and Kimura, 1981). Aristolochiaceae family members are widely used in traditional medicine and homeopathy (Lopes et al., 2001).

To meet the food and therapeutic needs of ever increasing population of human race, variety of means and methods are being adopted which include enhancing production in quality and quantity using high yielding seeds and through application of growth and yield increasing fertilizers. Among fertilizers, the bio fertilizers have great potential for supplying nutrients at the minimum energy utilization to agricultural crops and medicinal plants and they are cost effective too. The importance and necessity of use of such bio fertilizers in crop systems are also well established now (Subba, 1981). Use of biofertilizers is increasing very fast primarily to preserve the quality of soil for ecological reasons and to maintain soil fertility on a sustainable basis (Watanabe, 1986; Okon, 1988; Kaushik, 1985). Number of biofertilizers or microbial inoculants have been developed and enlisted so far (Tandon, 1992).

Effect of *P. indica* culture filtrate on economic and medicinal plants

Not only the fungal mycelium of *P. indica* forming symbiotic association that promoted plant growth but also culture filtrate of mycelium equally exerted positive effect on plant growth. The culture filtrate of the mycelium contains fungal exudates, hormones, enzymes, proteins, etc. A very small amount (50 µl) was sufficient to promote root as well as shoot growth in the culture tube experiment. In pot culture of plant however, 15 ml of freshly eluted culture filtrate was used for application to the pot. This resulted in increase of root length, shoot length and plant biomass in *P. indica* treated host plants, maize, bacopa and tobacco. Also similar observations were reported in culture tube experiments with the induction of secondary roots (Varma et al., 2001). This remains to be the first report of its kind showing positive impact of *P. indica* culture filtrate on promotion of plant growth.

These findings were further corroborated by the observations of Singh et al. (2003) who observed that culture filtrate of *P. indica* initially showed significant increase in neem and maize plant growth, and development over the control, however, the impact was steadily slowed down over the period of one year. Nevertheless, the plant height was invariably higher than those received AM fungi or none (Kumari, 2002; Kumari et al., 2004).

Potential of *P. indica* biomass for promoting growth of many plants (above 145 plants) has been documented so far. However, impact of *P. indica* culture filtrate on plant growth promotion has been studied only in few plants. The study makes a good addition to this novel aspect of *P. indica*.

In view of immense importance of *P. indica*, studies were carried out to investigate the effect of *P. indica* culture filtrate on the growth of medicinal plant, *A. elegans* and an alkaloid aristolochic acid in the plant extract with effect on growth and yield of oil content of economic plant, *H. annus* (Sunflower) as a model to reckon with. Also it involved studying conditions of bulk production of the fungus in fermenter to be formulated as biofertilizer for economic use. Characterization of *P. indica* by HPTLC analysis and quantitative analysis of microbial rhizospheric flora was also carried out (Bagde et al., 2010 a, b, c, d).

Impact of *P. indica* culture filtrate was studied in *H. annus* in greenhouse. Treatment with *P. indica* culture filtrate promoted overall growth of the plant in terms of increased, root collar diameter, number of secondary roots, root length, root weight, stem diameter, stem height, number of leaves, length and width of leaf, flower number, flower diameter, flower dry weight, number of seeds, weight of seeds and total biomass as compared to untreated control plants. Seed oil content considerably increased in treated plants (Bagde et al., 2010c).

Supplementation of culture filtrate in the substratum growing *A. elegans* Mart promoted overall biomass, rendered greener and improved leaf texture and improved active ingredient-aristolochic acid. In contrast, in unsupplemented control, the plant growth was stunted and foliage mass was reduced. Treatment of *A. elegans* plants with culture filtrate of *P. indica* exhibited significant increase in growth with respect to plant height, leaf diameter, length and dry weight, total biomass and aristolochic acid contents of the plants than untreated plants (Bagde et al., 2010d).

P. indica culture filtrate treated plants attained highest height, increased total biomass and important ingredients like oil and aristolochic acid as compared to untreated control plants. These effects may be due to production of addition of known and unknown compounds from culture filtrate that influenced plant development, with non-specific mechanism of interactions. There existed a positive phytopromotional correlation between addition of *P. indica* culture filtrate and *H. annus* and Aristolochia plants. Also confirmed by quantification of rhizospheric soil test, where it showed higher population of action-mycetes, bacteria and fungi. These results indicate the possibility of carbon drain efflux. Present study indicated that *P. indica* is quite efficient for increasing not only total biomass of the two plants studied but also medicinally and economically important ingredients such as seed oil in *H. annus* and aristolochic acid in *A. elegans*.

In order to get bulk culture of *P. indica* for formulation in commercially and economically cost effective bio fertilizer, it was cultivated in a 5 L capacity fermenter (New Brunswick, USA). The medium used was Aspergillus medium. *P. indica* was grown in Aspergillus medium which was the best medium for optimum growth as was evident by flask culture experiment with the organism. Various parameters of the fermentation were measured, maintained and recorded over the period of 20 days. It was observed that the pH of the medium in the range of 6.5 to 6.7 was more favourable for optimum growth of the organism. Temperature range of 25 to 35 was favourable for the growth of *P. indica* and not affected adversely. However, the temperature of 30°C was optimum and more favourable for optimum growth of *P. indica*. Oxygen requirement of the culture was fulfilled by forcing filtered air at 1.0 LPM concentration via sparger and it gave satisfactory results. Air bubbles were released in fine air bubble form and impeller distributed it efficiently throughout the growth medium. Baffles helped in process of agitation and avoided vortex formation. Growth proceeded is most obvious and satisfactorily mannered under the optimized conditions. Though there was short lag period, growth of the organism commenced soon as was evident by optical density readings taken subsequently after inoculation. Sporulation was observed in normal course due to optimization of growth conditions. The bulk culture of *P. indica* so obtained in fermenter was formulated into fertilizer (Bagde et al., 2010b).

Composition of Kaefer medium was the best growth medium for the optimum growth of *P. indica*. Other parameters required for optimum growth determined were pH (6.5), temperature (28°C), nutritional requirements (such as carbon from glucose, nitrogen from aspargine and peptone and yeast extract, potassium from KCL, and phosphorus from KH2PO4.

SDS-PAGE protein analysis of roots of two plants treated with *P. indica* culture filtrate showed qualitative and quantitative differences in polypeptides as compared to untreated control plants. A total of 7 and 12 protein bands were observed in control and *P. indica* treated *H. annus* (Sun gold) plants. At least 4 additional protein bands were absent in untreated control roots while these were prominently present in *P. indica* treated plants. As the amount of protein samples loaded in all the wells were identical, nature of bands in respect of fairness and prominence reflected up and down regulation of the protein. Approximate molecular weight determination of faint and prominent nature of the bands have shown that certain additional proteins were seen in *P. indica* treated plants that might be responsible for favourable growth development of the plant (Bagde, 2010).

In short, *P. indica*, a root colonizing fungus which is cultivable axenically, uniquely possesses multifunctional properties such as plant promoter, plant protector, resistance against heavy metals, bio herbicide, immune-modulator, resistance against temperature, salt tolerance as bio fertilizer and tool for basic research.

Mechanism of interaction of *P. indica* with plants

As per Sirrenberg et al. (2007) mechanism by which *P. indica* promotes the growth of plants is not yet very clear, although some studies have implicated various ingredients from *P. indica* or induced by it in plants for its positive effects, such as Indole-3-acetic acid (IAA) production in culture filtrate by *P. indica* or induction of IAA in plants, proteins in *P. indica* showing similarity to myrosinase binding and myrosinase associated proteins raising IAA that triggered growth promotion in plants. Leucin rich repeated protein production in plants, mediated by the endophyte, phytohormones, endophyte acting as modulator elevating nitrate assimilation and /or starch degradation in plants could be responsible for its positive impact on plants. Also improvement in the growth of the plant may be due to induction of systemic resistance by *P. indica* against diseases in the plants as has been reported by many researchers. The fungus has been sequenced by European scientists.

Sirrenberg et al. (2007) also contended that although mechanism by which *P. indica* improved the growth of plants was still unclear, at least in Arabidopsis, it is observed that the effect was due to diffusible factor that could be IAA, as *P. indica* produces IAA in culture filtrate. It is suggested that auxin production affecting root growth was responsible for or at least contributed to the beneficial effect of *P. indica* on its host plants. Sufficient quantities of IAA in culture filtrate of axenically grown *P. indica* were detected and it was observed that culture filtrate caused described root phenotype implicate production of auxin by the fungus. In addition, the fungus may induce auxin production in the plant (Peškan-Berghöfer et al., 2004).

Also in Arabidopsis, it was observed that cell wall extract from the fungus *P. indica* promoted growth of seedlings and induced intracellular calcium elevation in roots. The extract and the fungus also induced a set of genes in Arabidopsis roots including some with Ca^{2+} signaling related functions. The cell wall extracted induced cytosolic Ca^{2+}elevation in the roots of Arabidopsis and tobacco (Vadassery et al., 2009) and it was revealed that sequential cytoplasmic nuclear calcium elevations resulted in better plant performance when plant and fungus *P. indica* interacted. The beneficial interaction of Arabidopsis with growth promoting *P. indica* provides a nice model system to unravel signaling events to mutualistic or pathogenic plant/fungus interactions. Similarly in Arabidopsis, *P. indica* requires jasmonic acids signaling and cytoplasmic function of NRR1 to confer systemic resistance (Stein et al., 2009).

Serfling et al. (2007) evaluated the performance of *P. indica* in different substrata under greenhouse and field conditions and observed that *P. indica* colonization increased plant biomass in winter wheat. In greenhouse experiment, severity of the pathogen of leaf (*Blumeria. graminis*), stem (*Pseudocercosporella herpotrichoides*), and root (*Fusarium culmorum*) got reduced significantly, while in field experiments, symptoms caused by leaf pathogen did not differ in *P. indica* colonized plants as compared to untreated plants control. In the field, *P. herpotrichoides* disease severity was significantly reduced in plants colonized by the endophyte. In *P. indica,* colonized plants number of sheath layers and hydrogen peroxide concentration increased after *B. graminis* attack, suggesting that root colonization caused induction of systemic resistance or priming of the host plant.

Schäfer et al. (2009a) studied role of phytohormones in plant root, *P. indica* mutualism and stressed importance of phytohormones for compatibility in plant root *P. indica* associations. Schäfer et al. (2009b) studied molecular basis for barley root colonization with mutualistic *P. indica* by global gene expression profiling, metabolic profiling and genetic studies and implicated gibberellin as a modulator of the roots basal defence innate immunity. It is reported that *P. indica* contains substantial amounts of an acid phosphatase which has the potential to solubilise phosphate in the soil and delivers it to the plant (Malla et al., 2004). It was also demonstrated that growth promotion of Arabidopsis seedlings was associated with a massive uptake of phosphate from the growth medium (Shahollari et al., 2005).

Felle et al. (2009) observed that the fungus, *P. indica* induced fast root surface pH signaling and primed systemic alkalinization of the key apoplast powdery mildew infection in *Hordeum vulgare* L. and speculated that the primed pH increase was indicative of and supported the potential systemic response to *B. graminis* f.sp. hordeii induced by *P. indica*. Molitor et al. (2009) studied induced resistance triggered by *P. indica* and observed that two factors, plant hormone ethylene and proteobacterium Rhizobium radiobacter played role in the induced systemic resistance (ISR) response mediated by *P. indica*. *P. indica* mediates stress tolerance to colonized plants. However, it still has to be elucidated to what extent the host physiology is redirected by the endophyte to cause the effects mentioned (Schäfer et al., 2007). Yadav et al. (2010) performed cloning and the functional analysis of gene encoding phosphate transporter (PiPT) from *P. indica*. Expression of PiPT was localized to the external hyphae colonized with maize (*Zea mays)* plant root which suggested that external hyphae were the initial site of phosphate uptake from the soil. Higher amounts of phosphate was found in plants colonized with wild type *P. indica* than that of non colonized and plants colonized with KD-PiPT *P. indica* mutant. Observations suggested that PiPT is actively involved in the phosphate transformation and in turn *P. indica* helps in improvements of the nutritional status of the host plant.

When impact of *P. indica* on tomato growth and on interaction with fungal and viral pathogens was studied, it was observed that *P. indica* colonized the roots and improved the growth of tomato resulting in increased biomass of leaves by up to 20%. Limitation of disease severity caused by *Verticillium dahliae* by more than 30% was observed on tomato plants colonized by the endophyte. Also *P. indica* influenced the concentration of pepino mosoic virus in tomato shoots. Most importantly, the endophyte increases tomato fruit biomass (Fakhro et al., 2010). Nautiyal et al. (2010) studied interaction among *Paenibarthus lentimorbus, P. indica* and their consortia with native rhizobial population in the rhizosphere of *Cicer arietinum,* and reported that number of nodules and dry weight per plant was significantly enhanced. N, P, K uptake by plants was found to be maximum in *P. lentimorbus* treated plants followed by *P. indica* as compared with un-inoculated control plants. Treatments with *P. lentimorbus* and *P. indica* and their consortia significantly enhanced plant height in a 34.44, 19.73 and 35.32%, respectively; number of nodules in a 138.86, 94.09 and 133.95%, respectively; a dry weight in a 64.56, 44.0 and 53.16% respectively, as compared to un-inoculated plants. *P. indica* is a wide-host root colonizing endophytic fungus which allows the plants to grow under extreme physical and nutrient stress. It functions as a plant promoter, and biofertilizer in nutrient deficient soils, as a bioprotector against biotic and abiotic stresses including root, leaf pathogens and insect invaders, inducing early flowering, enhanced seed production and stimulation of active ingredients in medicinal plants. Positive increments are established for many plants of economic importance (Oelmüller et al., 2009).

Bagde et al. (2010 a, b, c, d) studied the impact of *P. indica*, a novel fungus on growth and yield of oil of commercially important plant *H. annus*. Also Impact of *P. indica* on growth and yield of aristolochic acid in *Aristolochia elegans*, a medicinally and important plant was studied. Optimum conditions for cultivation of *P. indica* was carried out, mass cultivation of *P. indica* in 5 L New Brunswick fermenter was carried out and formulation of *P. indica* biomass and culture filtrate in the form of bio fertilizer was carried out. Characterization of *P. Indica* culture filtrate by HPTLC was done, Electron microscopic study of *P. indica* was carried out. Biological spectrum of rhizospheric soils of two selected plants was carried out and molecular studies for protein profiling of *P. indica* and plant roots of plants under investigation were carried out (Bagde, 2010).

CONCLUSION

Potential of symbiotic fungus, *P. indica* cell mass for promoting growth of many medicinally and economically important plants has been documented so far. Also, impact of its culture filtrate on plant growth and its essential ingredients has been reported. Hence, Present investigation was carried out to characterize culture filtrate of *P. indica* by HPTLC analysis to ascertain mechanism of its action on plant growth. HPTLC analysis of *P. indica* culture filtrate revealed that it contains carbohydrates and saponins and in addition it may contain other ingredients of interest. Plant growth promotion effect of culture filtrate may be due to production or addition of known and unknown compounds from culture filtrate. It may contain many known and unknown constituents including hormones and enzymes, etc. This is confirmed by quantification of microorganisms in rhizospheric soil testing of plants, *H. annus* and *A. elegans* treated with culture filtrate of *P. indica* wherein it showed maximum number of bacteria, actinomycetes and fungi as compared to untreated plants (Bagde et al., 2010a).

FUTURE PROSPECTS OF *P. INDICA*

The high potential of multifaceted fungus, *P. indica* has tremendous applications in future as biofertilizer, protector and immunoregulator which will be helpful in improving quality of not only plants but also ultimately of food, nutrition, medicine and overall quality of human life. Also the fungus has been reported to possess good quantity of antioxidants. Mechanism by which *P. indica* promotes the growth of plants is unclear but some studies have implicated various factors induced by it in plants that were responsible for its positive effects. The continuous studies are bound to unravel yet unknown potential and properties of this fungus. In addition, it may have following prospects in future:

1. This investigation opens avenues for mass cultivation with value addition of economically important plant, Helianthus and medicinal plant Aristolochia with application of *P. indica* culture filtrate so as to increase commercial potential of the plants and their essential ingredients
2. New and novel fungus, *P. indica,* covers multidimensional characteristics which are not covered by any other existing genus. This study is a new feather in applications of its culture filtrate besides its already known applications of cells so far.

3. New fungus, *P. indica,* acts as a plant stimulator and pathogenic inhibitor. This opens new prospects for its application in agriculture, floriculture, viticulture and reclamation of degraded and heavily mined soils. Mechanisms of interactions of action of *P. indica* with biota like plants and animals and other microorganisms could be clearer in future
4. Avenues are opened to formulate *P. indica* into large scale production in fermenter to be formulated as bio fertilizer.
5. Unlike other fungi or AM fungi used as bio fertilizer, *P. indica and* AM like fungus can be used in form of culture filtrate for further development and formulation into a bio fertilizer.
6. HPTLC of culture filtrate of *P. indica* emphasizes importance of individual component of the culture filtrate involved in stimulation of growth of the plants.
7. There are prospects that ingredients present in culture filtrate, stimulated and produced in response to ingredients of culture filtrate in plants will be identified in future thereby opening more avenues of applications of *P. indica*.
8. *P. indica* is by now well established as bio fertilizer, bio protector, immunoregulator and agent for biological hardening of tissue cultured plants.
9. Many more properties and functions of *P. indica* cells and culture filtrate will be known in future

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